Patent Pending

User Guide

Version 1.2

Introduction

Realtime stain is a revolutionary dual use protein visualization and sample loading buffer specially formulated for SDS polyacrylamide gel electrophoresis (PAGE). **Real**time stain binds specifically to amine groups in proteins offering a unique alternative to the current post-staining techniques. Simply add the **Real**time stain to your protein sample (in a 1:1 ratio) and heat then watch your protein visibly running directly down the gel in **Real**time. The simple protocol offers you the flexibility to optimize and customize the labelling efficiency with the capability to generate your own pre-stained molecular weight standards. **Real**time stain is currently optimized for pure and partially-pure protein samples, recombinant protein fragments, FPLC fractions and antibodies.

Storage conditions & shelf life

Realtime stain should be stored at room temperature (18-24°C) and used within one year of the production date (see expiry date for details). Solution may solidify and require warming (30-40°C for 30-60seconds) prior to use if stored at a lower temperature.

Lyophilized Bovine serum albumin (BSA) should be stored at room temperature and used within the expiry date. When resuspended, the BSA (25mg/ml) should be aliquoted and stored at -20°C.

Contents

- 2ml or 0.2ml **Real**time stain solution (blue cap)
- 5mg Bovine serum albumin (red cap)
- 1x White screen (10x10cm gel)
- 1x White screen (8x10cm gel)
- 1x Vivaspin 20, 10,000Da MWCO (2ml **Real**time stain only)
- 1x User Guide
- 1x Easy Guide

Protocol requirements (not supplied)

- Dry heat block
- Reducing agent (step-1 and step-2)
- Microcentrifuge
- Refrigerated bench top centrifuge (for custom molecular weight standards only)
- Sample loading buffer (for custom molecular weight standards only)
- SDS PAGE gel system

Procedures

Realtime stain is formulated as a 2x solution. Simply add the **Real**time stain to your sample in a 1:1 ratio (5 + 5µl is recommended for most applications) then follow the protocol according to the amount of reducing agent required. White screens are supplied to aid visualization during running. See fitting instructions for details.

Protocol: Standard

Step-1: non-reduced or <10mM BME/DTT

1. Add 5µl **Real**time stain to 5µl sample
   (add up to 10mM BME or DTT if required)
2. Heat for 10minutes at 100°C
   (Quick pulse in a microfuge)

Step-2: optional for >10mM BME/DTT

3. Add required amount of reducing agent
   (0.5µl of 1-2M BME or DTT final concentration 50-100mM)
4. Heat for additional 3minutes at 100°C
   (Quick pulse in a microfuge)

Load and run sample

5. Load 5µl on to SDS PAGE gel
6. Run gel according to manufacturer's instructions

Note: Labelling efficiency can be optimised by varying the time and temperature of 'step-1' if required. See 'performance and & technical data' for more information.

Note: Gels can also be post-stained with Quick Coomassie, if required.

Buffer compatibility testing

5mg of BSA is supplied for direct buffer compatibility testing and to serve as a positive control for your SDS PAGE. Some amine containing buffers can affect labelling efficiency (see compatibility table 1 below for further details). If you do not know your buffer composition or your buffer is likely to contain incompatible components then Generon recommends using the following protocol for buffer testing prior to sample use:

1. Resuspend 5mg BSA in 200µl deionised water (final concentration 25mg/ml)
   (Note: once dissolved, aliquot and store at -20°C for future use)
2. Add 1µl of 25mg/ml BSA to 9µl buffer, and 10µl **Real**time stain. Repeat with PBS or 20mM Tris pH 7.5
3. Heat for 10minutes at 100°C
   (Quick pulse in a microcentrifuge)
4. Load 4µl on to an SDS-PAGE gel

Any incompatible components will significantly inhibit labelling efficiency and reduce BSA band intensity. If present, then we recommend diluting out 2-5 fold with deionised water or a compatible buffer, or remove the incompatible component via buffer exchanging prior to using the **Real**time stain. Re-run the protocol using a dilution series if required.

Table1. Realtime buffer compatibility.

Buffer component	Conc.	Compatible?	Conc.	Compatible?
Tris	<50mM	✓	>100mM	✗
NaCl	0.5M	✓		
BME & DTT	<10mM (Step-1)	✓	>10mM (Step-2)	✓
TCEP	<5mM (Step-1)	✓	>5mM (Step-2)	✗
Imidazole	<50mM	✓	>100mM	✗
Urea	4M	✓		
Glycine	50mM	✗		

Protocol: Molecular weight standards

Designed for the production of customised pre-stained molecular weight standards and ladders. Labelling efficiency should be tested using the standard protocol above prior to molecular weight standard production.

1. Add 20µl protein to 20µl **Real**time stain,
   (Add up to 10mM BME or DTT, if required)
   repeat separately for each individual protein standard
2. Heat for 10minutes at 100°C
   (Quick pulse in a microcentrifuge)
3. Combine the protein samples together in equal ratio
4. Buffer exchange with 20mM Tris pH 7.5 or PBS pH 7.4 using a Vivaspin 20, 10,000Da MWCO (supplied with 2ml **Real**time stain) and a refrigerated bench top centrifuge at 4°C
   (200 fold or 2x5ml buffer exchanges are required, with a final volume equal to the initial protein start volume: 20µl)
5. Add sample loading buffer (not supplied)
   (Note: once made, aliquot and store at -20°C for future use)
6. Heat 5-10µl for 3minutes at 100°C
   (Quick pulse in a microcentrifuge)
7. Load 5-10µl on to an SDS PAGE gel

For the best results: label each protein individually before combining in equal ratio and removing the unreactive dye. Aim for a final protein concentration, after buffer exchange, of 1-2mg/ml per protein.

Note: This protocol is designed for removing unreactive dye from the protein solution, reducing the running dye front, and can be used for small (<10-15kDa) proteins which would normally be masked by the dye front.

Note: Standards made using this protocol are compatible with all SDS PAGE gel types if compatible sample loading buffers and SDS PAGE gels are used.

SDS polyacrylamide gel compatibility

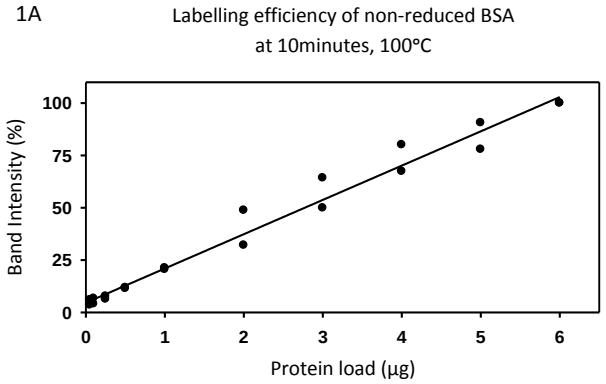
Realtime stain has been tested with Tris-Glycine, Tris-HEPES, and Bis-Tris pre-cast and cast SDS polyacrylamide protein gels. We are not currently aware of any issues with other gel types not described above, however, these have not currently been tested using the **Real**time stain.

Performance & technical Data

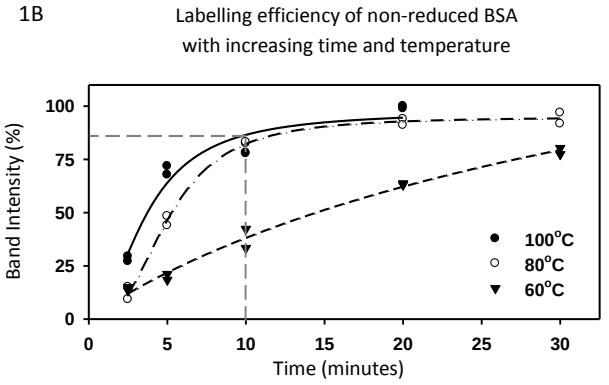
Labelling efficiency can be optimized for individual proteins by varying the labelling parameters (time, temperature, and reducing agent). Figure 1 shows performance data generated using the supplied BSA control.

Figures 1A-D: Labelling efficiency of the **Real**time stain with BSA. All samples were prepared in accordance with the protocol and run on NuSep 4-20% Tris-Glycine SDS PAGE pre-cast gels at 170volts (5µg BSA load unless otherwise stated). Each gel was dried and scanned using an over-head light source and the band intensity measured using Image J. (A) Non-reduced labelling efficiency at 10minutes, 100°C. (B) Non-reduced labelling efficiency with increasing time and temperature, recommended 10minute, 100°C labelling highlighted. (C) Effect of reducing agents upon step-1 labelling efficiency at 10minutes, 100°C. (D) Effect of 50mM reducing agent upon step-1 and step-2 labelling efficiency.

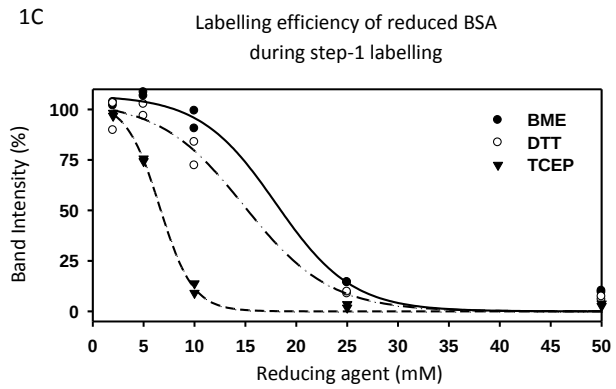
1A



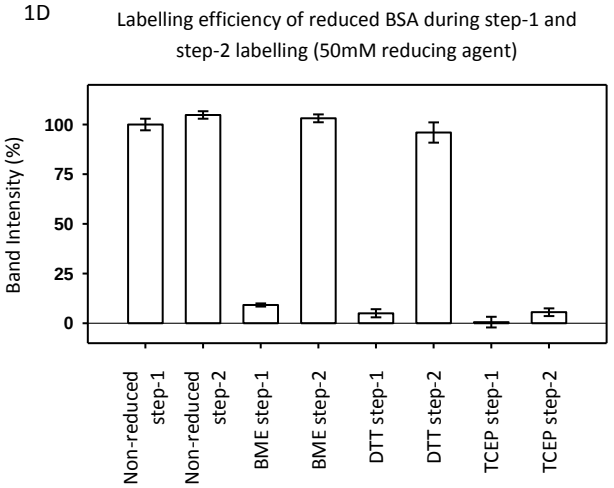
1B



1C



1D



White screen: Fitting instructions

Optional 8x10cm and 10x10cm gel white screens can be used to aid visibility during electrophoresis. Fitting instructions:

Life Technologies XCell SureLock® Mini-Cell: Setup the gel and tank as per manufacturer's instructions. Then simply slide the appropriately sized white screen (8x10 or 10x10cm gel) in-between the gel cassette and the electrode assembly (1) prior to loading the samples (figure 2).

Bio-Rad mini-PROTEAN: Place the 8x10cm gel white screen on to the electrode assembly (1), so it sits within the gasket seal. Then lock the gel cassette into place (2) taking care not to disrupt the gasket seal in the process (figure 3).

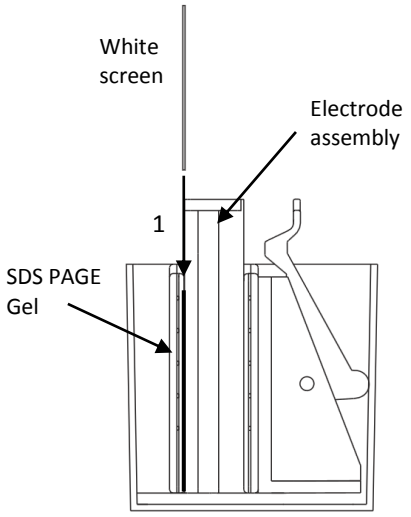


Figure 2. Insertion procedure for the 8x10cm or 10x10cm gel white screen into the *XCell SureLock® Mini-Cell tank* (image courtesy of *Life Technologies*).

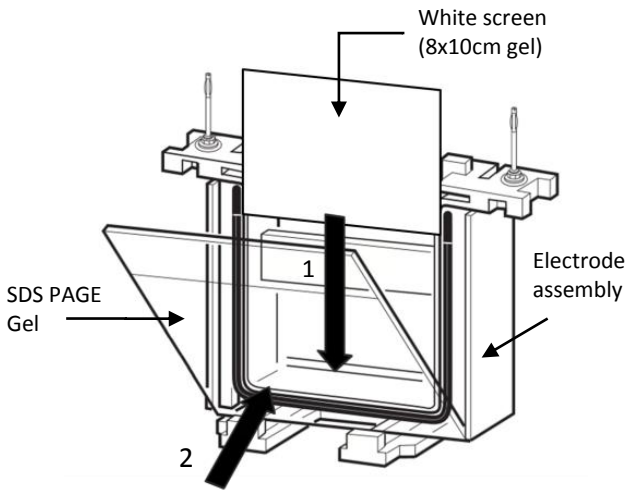


Figure 3. Insertion procedure for the 8x10cm gel white screen into the *Bio-Rad mini-PROTEAN* tank (image courtesy of *Bio-Rad*).

Troubleshooting assistance

Problem	Possible Cause	Solution
No visible or very faint bands	>10mM reducing agent used during step-1 labelling	Add reducing agent at step-2 (see protocol and figures 1C and 1D for details)
	>5mM TCEP reducing agent used during step-1 labelling	See figure 1C and 1D for performance details. Recommend using BME or DTT at the appropriate step
	>50mM TCEP reducing agent used during step-2 heating	Use a compatible reducing agent such as BME or DTT. See figure 1D for details
	Low protein concentration	Increase sample loading volume to 10µl
		Optimize staining by increasing the labelling time (see figure 1B)
		Concentrate protein sample prior to adding the **Real**time stain
	Limited availability of amine groups in the protein	Increase the labelling time for proteins with low numbers of Arginine and Lysine residues to compensate
	Sample dried out during labelling. Can occur when using extended labelling times and small sample volumes	Increase the volume of sample for longer labelling times. For example: add 20µl protein sample to 20µl **Real**time stain when labelling for 10minutes, 100°C
	Incompatible buffer component. See table 1 for details	Test for incompatible components using the 'buffer compatibility test'
		Dilute out the incompatible component (2-5 times) or buffer exchange out the component before use
	Possible heat block failure	Check the temperature of the heat block
Unable to view low molecular weight protein (<10-15kDa)	Protein runs within the dye front	Use the 'molecular weight standards' protocol to remove excess stain
Broad dye front	Large load volume (>10µl)	Reduce the load volume to <10µl. Increase concentration of the protein if required
	Continual heating of **Real**time stain at high temperatures (100°C) prior to use causes the dye front to broaden	Non-recoverable effect caused by the heating up of the **Real**time stain

Troubleshooting assistance continued …

Problem	Possible Cause	Solution
No visible or very faint bands seen with the BSA control during 'buffer compatibility testing'	Possible heat block failure	Check the temperature of the heat block
	Excess freezing and thawing of the stock solution	Purchase new BSA control sample. See 'ordering information' for further details
	Incompatible resuspension buffer	Purchase new BSA control sample. See 'ordering information' for further details. Resuspend in deionised water or compatible buffer (PBS pH 7.4 or 20mM Tris pH 7.5)
Poor band resolution of custom made molecular weight standards	Heating of the sample during centrifugation	Repeat using a refrigerated bench top centrifuge at 4°C
	Poor labelling efficiency	Optimize labelling efficiency (time and temperature) using the 'standard' protocol prior to making the standards
	Incompatible buffer component	Test for incompatible components using the 'buffer compatibility test'
Solidified **Real**time stain	Low temperature storage (2-4°C)	Warm **Real**time stain gently (30-40°C) for a short period and mix well before use. It is not recommended to heat **Real**time stain above this prior to use
Poor definition of lysate/serum bands	Not currently optimized for use with complex protein mixtures	Use standard sample loading buffer and stain with Quick Coomassie

Technical Support

For the latest technical support information:

- Go to www.generon.co.uk and search for '**Real**time stain'
- Submit a question to our support team at info@generon.co.uk
- Or contact our telephone sales and support team on +44 (0)1753 866-511

Materials Safety Data Sheet (MSDS)

Materials Safety Data Sheets are available from Generon upon request.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available upon request from Generon by quoting the product catalogue number and lot number printed on the tin.

Ordering Information

Product	Order number
2ml **Real**time stain	GEN-RT-STAIN-2000
0.2ml **Real**time stain	GEN-RT-STAIN-200
White screen (10x10cm gel)	GEN-RT-SCREEN-1010
White screen (8x10cm gel)	GEN-RT-SCREEN-810
Vivaspin 20, 10,000Da MWCO (12 pack)	VS2001
10g Bovine Serum Albumin (>99%)	GEN-BSA-10
1g Bovine Serum Albumin (>99%)	GEN-BSA-1

Disclaimer

This product is designed for research purposes only. No right to resell this product or any of its components is conveyed. Please contact Generon Ltd for further information.

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